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Step I - First Strand Synthesis

m⁷G ————— (A)_n Messenger RNA

↓ Annealing of oligo d(T) Primer

m⁷G ————— (A)_n
— (dT)

↓ Reverse Transcriptase

m⁷G ————— (A)_n
— (dT)

Step II - Second Strand Synthesis

DNA Polymerase I
Ribonuclease H
E. coli DNA Ligase

Alkaline Hydrolysis of RNA
Terminal Transferase
dTTP

————— (dA)_n TTTTTT ————— (dT)_n
————— (dT)

↓ Oligo d(A)_n
T7 DNA Polymerase

AAAAAA —————
TTTTTT ————— (dT)_n

T4 DNA Polymerase

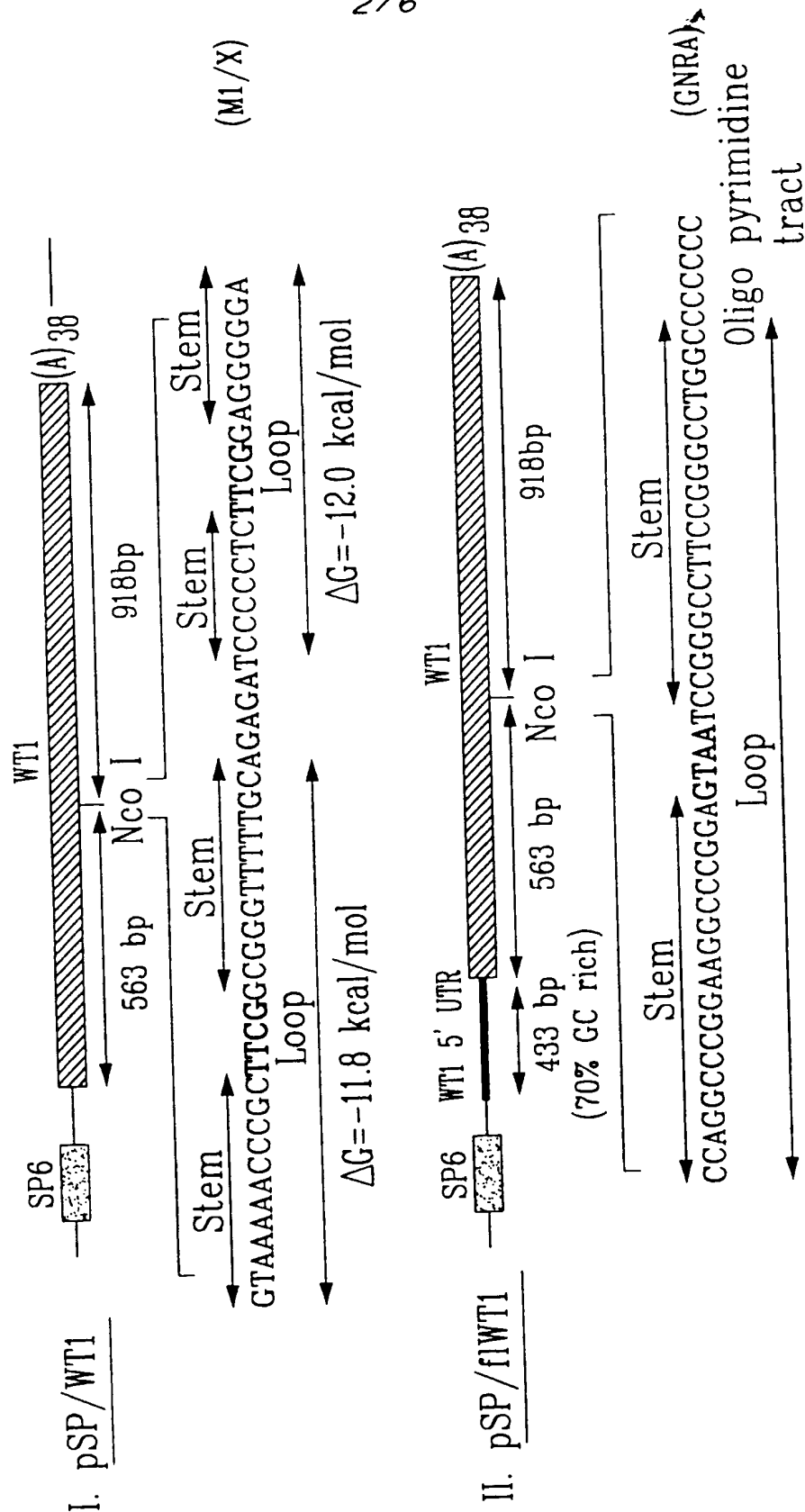
T4 Ligase
(Adaptors)

————— (dA)
————— (dT)

Step III - Polishing and Cloning

Clone into appropriately
prepared vector

FIG. 1 PRIOR ART



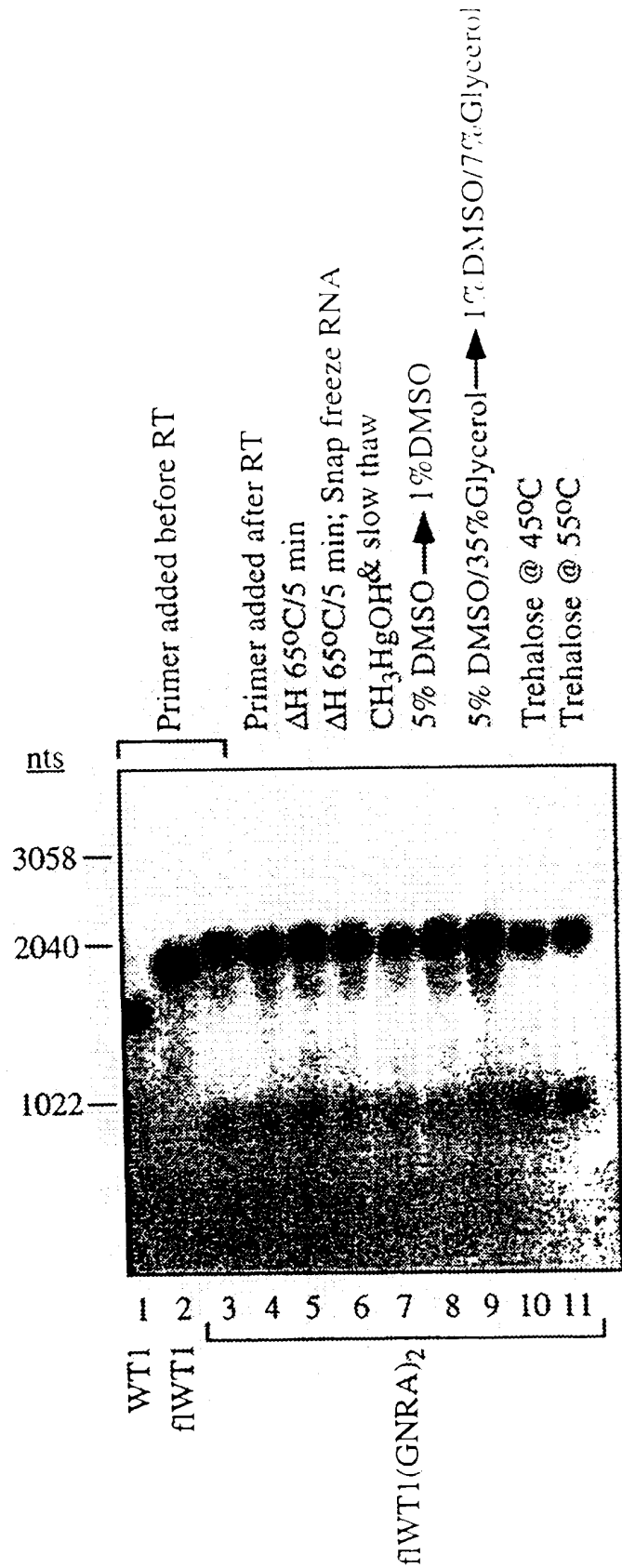
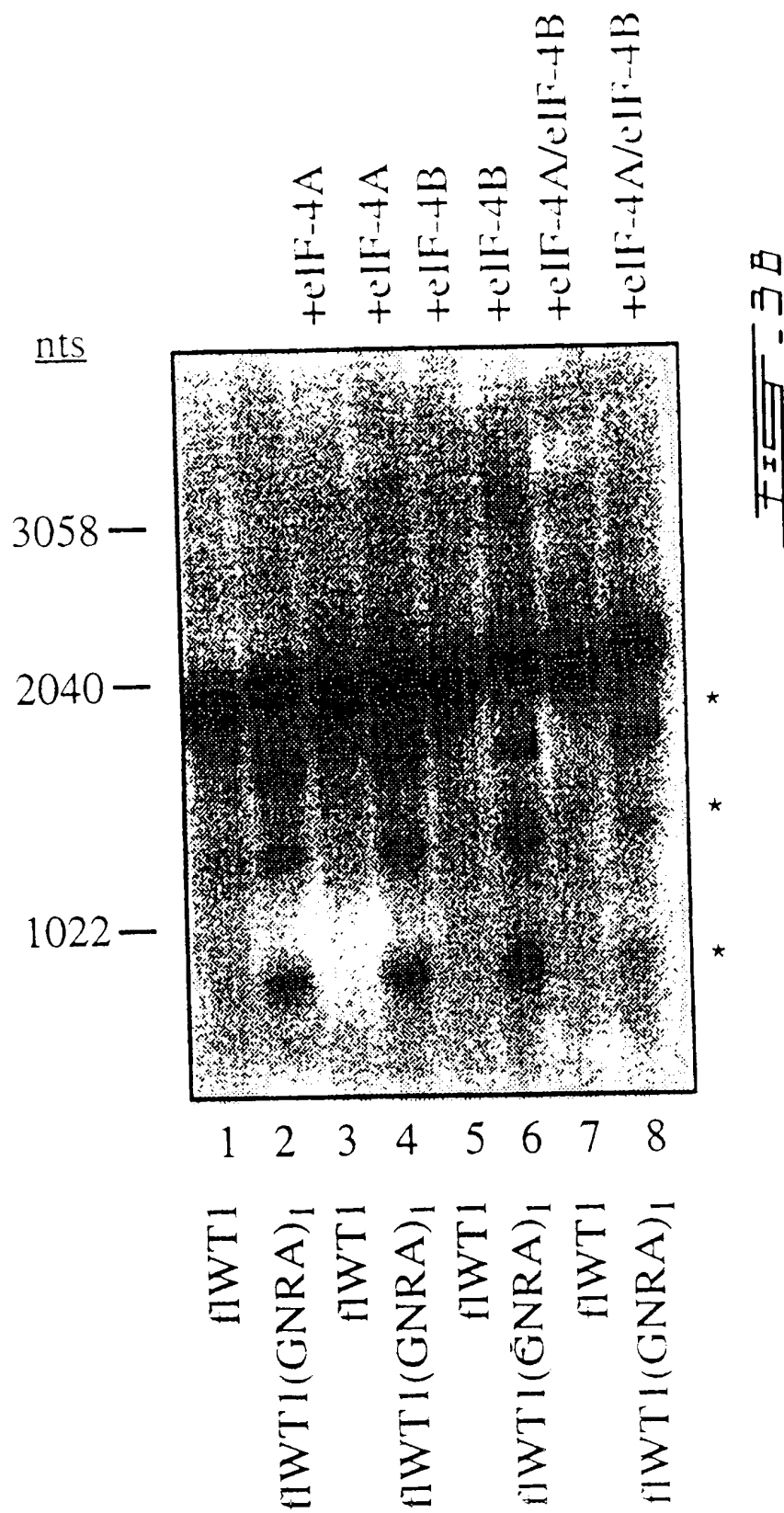


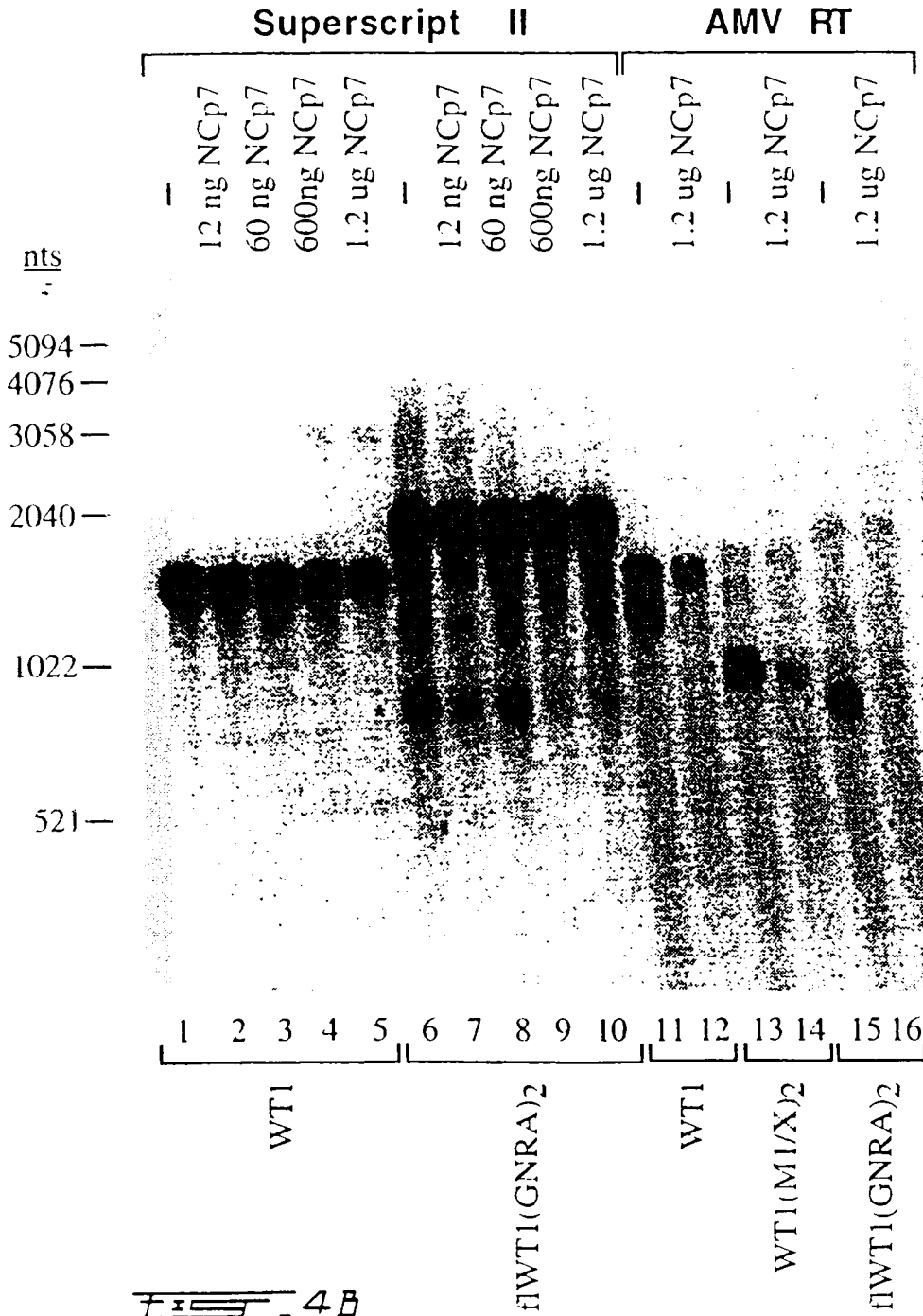
FIG. 3A



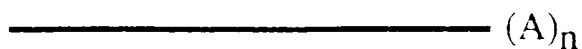
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NH₂-MQRGNFRNQRKNVKCFNCGKEGHTARNCRAPRKKGCWKCCKEGHQMKDCTERQANFLGKIWPSYKGRPGNFL-COOH

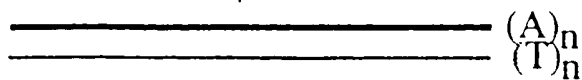
FIG. 4A



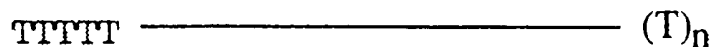
In vitro transcribed WT1 mRNA



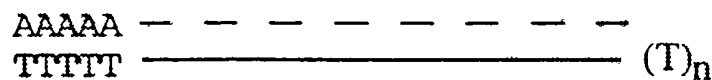
↓ Superscript II
Oligo d(T)_n



↓ Alkaline hydrolysis of RNA
Terminal transferase
dTTP



↓ T7 DNA polymerase
Oligo d(A)_n
α-³²P-dATP



Alkaline agarose gel Analysis

Fraser - 5A

